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(21) International Application Number: PCT/US97/04536 (22) International Filing Date: 18 March 1997 (18.03.97) (30) Priority Data: 08/693,577 7 August 1996 (07.08.96) US (71) Applicant: WISCONSIN ALUMNI RESEARCH FOUNDATION [US/US]; P.O. Box 7365, Madison, WI 53707-7365 (US). (72) Inventors: COOK, Mark, E.; 15 Kewaunee Court, Madison, WI 53705 (US). PARIZA, Michael, W.; 7102 Valhalla Trail, Madison, WI 53719 (US). (74) Agent: KRYSHAK, Thad; Quarles & Brady, 411 East Wisconsin Avenue, Milwaukee, WI 53202-4497 (US).	(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>	
(54) Title: CONJUGATED LINOLEIC ACID TO MAINTAIN OR ENHANCE BONE MINERAL CONTENT		
(57) Abstract A method of treating an animal to maintain or enhance the mineral content of the bones of the animal consists of administering to the animal a safe and effective amount of C.I.A. Compositions for use in the method are also disclosed.		

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CONJUGATED LINOLEIC ACID TO MAINTAIN OR ENHANCE BONE MINERAL CONTENT

Field of the Invention

The present application generally relates to methods of treating animals, including humans. More particularly, it relates to methods of treating animals to maintain or enhance the mineral content of bone.

Background of the Invention

It is known that many animals, including humans, lose bone minerals as they age or if they are afflicted with certain diseases. Researchers have observed that bone calcium loss or reduction in humans can cause osteoporosis. It also is known that maintaining or enhancing the bone calcium content can be beneficial in treating or preventing osteoporosis.

Most of the known chemical compounds that maintain or increase the bone calcium in the animal are potent biological agents which can have significant side effects.

It would be advantageous to have methods and compositions for maintaining or enhancing the mineral content of skeletal bones in animals without causing significant undesirable side effects.

Brief Summary of the Invention

It is one object of the present invention to disclose a method of maintaining or enhancing the mineral content of skeletal bones in animals.

It is a still further object to disclose novel compositions for use in the methods of the invention.

We have discovered a method of maintaining or enhancing the mineral content of skeletal bones in an animal, including a human, which comprises administering to the animal a safe and effective amount of a conjugated linoleic acid, such as 9,11-octadecadienoic acid and 10,12-octadecadienoic acid, or active derivatives thereof, such as non-toxic salts, active esters, such as triglycerides, and mixtures thereof.

The conjugated linoleic acids, their non-toxic salts, active esters, active isomers, active metabolics, and mixtures thereof are referred to herein as "CLA."

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The compositions of the present invention are pharmaceutical compositions which contain CLA in combination with a source of calcium, such as calcium carbonate, and optionally other ingredients.

5 It will be apparent to those skilled in the art that the forementioned objects and other advantages may be achieved by the practice of the present invention.

Description of the Preferred Embodiment

10 In the preferred method of the present invention for maintaining or increasing the mineral content of skeletal bones of an animal, a safe and effective amount of CLA is administered to the animal.

Although CLA can be administered by itself to enhance or maintain bone mineral content, the preferred compositions of the
15 present invention are pharmaceutical compositions which contain safe amounts of CLA and a calcium source, such as calcium carbonate, calcium phosphate or calcium carbonate. The compositions may also contain, without limitation, pharmaceutical diluents and other ingredients including
20 vitamins, such as Vitamin D, and other minerals, such as zinc and magnesium salts. The pharmaceutical composition may be in the form of tablets, capsules or liquids for oral administration. Since CLA is a natural food ingredient and it is relatively non-toxic, the amount of CLA which can be
25 administered is not critical as long as it is enough to be effective.

The practice of the present invention is further illustrated by the examples which follow:

Example 1

30 Day old broiler chicks were fed a conventional corn/soybean meal diet containing either 0.5% corn oil (control) or 0.5% CLA. The chicks were fed the diets for 27 days. The chicks were then sacrificed and the bones were collected, dried, ether extracted and the ash content percentage was determined using a muffle
35 furnace. The results of percent ash of fat-free, dried bone and of bone as collected are shown in Table 1. The body weights and results of bone evaluation are shown in Table 2.

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Table 1:

Diet	% Moisture	% Fat	% Ash (fresh weight)	%Ash
Control	44.90±0.93	3.94±0.44	20.46±0.73	51.11±1.74
CLA	46.22±1.05	4.18±0.43	23.28±1.13	56.32±2.10

Table 2:

Diet	Body Wt	Shank Width mm	Tibia Width mm	Tibia Length cm	TD* Score
Control	737.5±31.41	8.0±0.42	6.07±0.23	6.91±0.11	1.6±0.31
CLA	830.9±29.66	8.43±0.23	5.91±0.20	7.09±0.09	1.3±0.15

*TD = tibial dyschondroplasia. Growth plates of the tibia were examined for abnormal cartilage accumulation and scored 1 if normal, 2 if slightly enlarged, 3 if moderately enlarged, and 4 if severely enlarged.

The results of the experiments demonstrate that the bones of the chicks fed the diet containing CLA have a higher ash and mineral content than the control chicks. Similar results are obtained in humans.

5 The methods of the present invention may take several embodiments. In one embodiment, the CLA is administered to an animal in a pharmaceutical or veterinary composition containing a safe and effective dose of the CLA. In another embodiment, the animal is fed a food that has been enriched with CLA.

10 The animal feeds and pharmaceutical preparations for use in the methods of the present invention are those containing the CLA in combination with a conventional animal feed (e.g. poultry feed), human food supplement, or approved pharmaceutical diluent.

15 Active forms of CLA include the free conjugated linoleic acids, the active isomers of those acids, non-toxic salts thereof, active esters of those acids, such as the triglycerides, methyl and ethyl esters, and other active chemical derivatives thereof, and mixtures thereof.

20 The free conjugated linoleic acids (CLA) have been previously isolated from fried meats and described as anti-carcinogens by Y. L. Ha, N. K. Grimm and M. W. Pariza, in *Carcinogenesis*, Vol. 8, No. 12, pp. 1881-1887 (1987). Since

then, they have been found in some processed cheese products (Y.L. Ha, N. K. Grimm and M. W. Pariza, in *J. Agric. Food Chem.*, Vol. 37, No. 1, pp. 75-81 (1987)).

5 The free acid forms of the CLA may be prepared by isomerizing linoleic acid. The non-toxic salts of the free CLA acids may be made by reacting the free acids with a non-toxic base. Natural CLA may also be prepared from linoleic acid by the action of Δ^{12} -cis, Δ^{11} -transisomerase from a harmless microorganism such as the Rumen bacterium Butyrivibrio
10 fibrisolvens. Harmless microorganisms in the intestinal tracts of rats and other monogastric animals may also convert linoleic acid to CLA (S.F. Chin, W. Liu, K. Albright and M.W. Pariza, 1992, *FASEB J.* 6:Abstract #2665).

The CLA obtained by the practice of the described methods
15 of preparation contains one or more of the 9,11-octadecadienoic acids and/or 10,12-octadecadienoic acids and active isomers thereof. It may be free or bound chemically through ester linkages. The CLA is heat stable and can be used as is, or dried and powdered. The free acids are readily converted into
20 a non-toxic salt, such as the sodium, potassium or calcium salts, by reacting chemically equivalent amounts of the free acid with an alkali hydroxide at a pH of about 8 to 9.

Theoretically, eight possible geometric isomers of 9,11- and 10,12-octadecadienoic acid (c9,c11; c9,t11; t9,c11; t9,t11;
25 c10,c12; c10,t12; t10,c12 and t10,t12) would form from the isomerization of c9,c12-octadecadienoic acid. As a result of the isomerization, only four isomers (c9,c11; c9,t11; t10,c12; and c10,c12) would be expected. However, of the four isomers, c9,t11- and t10,c12- isomers are predominantly produced during
30 the autoxidation or alkali-isomerization of c9,c12-linoleic acid due to the co-planar characteristics of 5 carbon atoms around a conjugated double-bond and spatial conflict of the resonance radical. The remaining two c,c-isomers are minor contributors.

The relatively higher distribution of the t,t-isomers of
35 9,11- or 10,12-octadecadienoic acid apparently results from the further stabilization of c9,t11- or t10,c12-geometric isomers, which is thermodynamically preferred, during an extended processing time or long aging period. Additionally

the t,t-isomer of 9,11- or 10,12-octadecadienoic acid that was predominantly formed during the isomerization of linoleic acid geometrical isomers (t9,t12-, c9,t12- and t9,c12-octadecadienoic acid) may influence the final ratio of the isomers or the final
5 CLA content in the samples.

Linoleic acid geometrical isomers also influence the distribution of minor contributors (c,c-isomers of 9,11- and 10,12-, t9,c11- and c11,t12-octadecadienoic acids). The 11,13-isomer might be produced as a minor product from
10 c9,c1-octadecadienoic acid or from its isomeric forms during processing.

The CLA, in addition to being added to an animal's food, can be administered in the form of pharmaceutical or veterinary compositions, such as tablets, capsules, solutions or emulsions
15 to the animal or human. The exact amount to be administered, of course, depends upon the form of CLA employed and the route of administration, and the nature of the animal's or human's condition. Generally, the amount employed of CLA employed as a pharmaceutical will range from about one part per million (ppm)
20 to about 10,000 ppm of CLA (calculated as the free acids) in the human's diet. However, the upper limit of the amount to be employed is not critical because CLA is relatively non-toxic. The amounts of CLA (calculated as the free acids) to be added to food as an additive can range from .01% to 2.0% or more by
25 weight of the food.

The preferred pharmaceutical compositions of the present invention contain the non-toxic CLA sodium, potassium, or calcium salts in combination with a calcium source and pharmaceutical diluents. When the compositions are intended for
30 oral administration the dosage forms preferably will be a tablet, a wafer, a capsule or a liquid. When the compositions are solutions or suspensions intended for parenteral administration, the preferred diluent will be Sterile Water for Injection U.S.P.

35 The calcium sources that may be used in the compositions of the present invention include, without limitation, salts, such as calcium carbonate, calcium gluconate and calcium phosphate. Especially preferred is dibasic calcium phosphate.

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A representative pharmaceutical tablet has the following formula:

- 5 CLA (calculated as free acids) 600 mg
 Dibasic Calcium Phosphate,
 (anhydrous form) 500mg
 Cholecalciferol (133 IU) 3.33 mcg
 Microcrystalline cellulose, sodium starch glycolate,
 corn starch, hydrogenated vegetable oil wax, magnesium
 stearate and talc added.

- 10 The normal daily dosage to increase or maintain proper bone
 calcium levels is three to thirty tablets per day. Thirty tablets
 a day is roughly equivalent to the dose of CLA used in Example 1.

A representative chewable pharmaceutical wafer has the following formula:

- 15 CLA (calculated as free acids) 1000 mg
 Cholecalciferol 5 mcg (200 IU)
 Dibasic Calcium Phosphate, (anhydrous form)
 Added dextrose, sucrose, talc, stearic acid, mineral
 oil, salt, and natural and artificial flavorings.

- 20 The normal daily dosage is two to twenty tablets a day.
 The twenty tablets a day is roughly equivalent to the amount of
 CLA used in Example 1.

- Because CLA and calcium salts are relatively non-toxic,
 doses larger than the normal daily dosages of the compositions
25 may be administered if desired.

 It will be readily apparent to those skilled in the art
 that a number of modifications or changes may be made without
 departing from the spirit and scope of the present invention.
 Therefore, the invention is only to be limited by the claims.

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We claim:

1. A method of maintaining or enhancing the mineral content of bone in an animal, the method comprising administering orally or parenterally to the animal a safe amount of CLA, the amount being effective to maintain or enhance the mineral content.
2. The method of claim 1 in which the CLA is a triglyceride of a conjugated linoleic acid.
3. The method of claim 1 in which the CLA is a sodium salt of a conjugated linoleic acid.
4. The method of claim 1 in which the CLA is a potassium salt of a conjugated linoleic acid.
5. The method of claim 1 in which the CLA is a calcium salt of a conjugated linoleic acid.
6. The method of claim 1 in which the CLA is 9,11-octadecadienoic acid.
7. The method of claim 1 in which the CLA is 10,12-octadecadienoic acid.
8. A pharmaceutical composition for maintaining and enhancing bone calcium content comprising safe amounts of CLA and a calcium source.
9. A pharmaceutical composition of claim 8 in which the calcium source is dibasic calcium phosphate.
10. A pharmaceutical composition of claim 8 in which the calcium source is calcium carbonate.
11. A method of maintaining or enhancing the calcium content of bone in an animal, the method comprising administering orally or parenterally to said animal safe amounts of a calcium source and CLA, said amounts being effective to maintain or enhance the calcium content of bone in the animal.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/04536

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/20 //(A61K31/20,A61K33:06,33:10)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 585 057 A (SCOTIA HOLDINGS PLC) 2 March 1994	1,3
Y	see page 2, line 1 - line 13 See page 4 lines 9-20, lines 40-43 and lines 49-56	2,4,5
Y	EP 0 585 026 A (SCOTIA HOLDINGS PLC) 2 March 1994	1-5
A	see the whole document	1,3
A	EP 0 261 814 A (EFAMOL LIMITED) 30 March 1988	8,9
	see the whole document	
	-/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

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Date of the actual completion of the international search

4 July 1997

Date of mailing of the international search report

16.07.97

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INTERNATIONAL SEARCH REPORT

Intern. Patent Application No
PCT/US 97/04536

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,Y	<p>WO 96 34855 A (SCOTIA HLODINGS) 7 November 1996 see pages 8,9,19,21,23-24 -----</p>	1-5

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 97/04536

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim(s) 1-7,11
is(are) directed to a method of treatment of the human/animal
body, the search has been carried out and based on the alleged
effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 97/04536

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